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(54) Title: <b>UROGENITAL AND INTESTINAL COMPOSITIONS</b>			
(57) Abstract  This application relates to compositions useful in preventing and/or treating urogenital and intestinal disorders, comprising an effective amount of at least one plant species of the Ericaceae family or its extract and a culture of at least one species of microencapsulated bacteria selected from the group consisting of lactobacillus, bifidobacterium and mixtures thereof.			

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## UROGENITAL AND INTESTINAL COMPOSITIONS

5

TECHNICAL FIELD

This application relates to compositions useful in preventing and/or treating urogenital and intestinal disorders.

BACKGROUND OF THE INVENTION

10       Complex, microscopic ecosystems pervade the urogenital and intestinal tracts of warm blooded animals. Trillions of microorganisms, comprising hundreds of species, occupy the urogenital and intestinal tracts of mammals, influencing and maintaining digestive and urologic functions. Microorganisms occupying these regions range from potentially pathogenic strains, such as *Escherichia coli*, enterococci, candida, gardnerella,  
15       klebsiella and clostridia, to the relatively nonpathogenic, such as lactobacillus and bifidobacterium. Deviations from this delicate flora balance have been etiologically linked to a number of urogenital and/or gastrointestinal tract disorders; such imbalances usually resulting in the proliferation and predominance of pathogenic species. Establishing and/or preserving such a delicate flora balance is, therefore, essential to maintaining optimal  
20       health.

One way of establishing or maintaining the body's flora is by administering lactobacillus. The use of lactobacillus to treat urogenital and intestinal disorders has been proposed, for instance, in Canadian Patent 1,298,556, issued April 4, 1992, to Bruce et al., PCT Application Serial Number WO 93/09793, published May 27, 1993, to Reid et al. and  
25       U.S. Patent 5,176,911, issued January 5, 1995, to Tosi et al. Researchers hypothesize that lactobacillus provide host protection via adherence to intestinal or vaginal epithelial cells.

Notwithstanding such proposals, several factors have been identified, casting doubt on the use of lactobacillus as single agent therapy for urogenital and/or gastrointestinal infection. 1) Variation exists among strains of lactobacillus concerning the degree to  
30       which individual strains of lactobacillus can adhere to epithelial surfaces. Reid, G. et al., Examination of strains of Lactobacillus for properties that may influence bacterial interference in the urinary tract, J. Urol., 138:330-335;1987. This variation may also extend to individual microorganisms. 2) Pathogenic microorganisms are most reliably excluded where lactobacillus colonies are established *prior to* pathogenic invasion.  
35       Hawthorn, L.A. et al., Exclusion of uropathogen adhesion to polymer surfaces by Lactobacillus acidophilus, Jour. Biomed. Mat. Res., 24:39-46;1990; a nonexistent

situation, however, barring *in vitro* design. 3) Viability issues exist regarding orally ingested live bacterial cultures. Clinical research reveals that not all live bacteria survive the digestive fluids of the stomach and upper intestinal tract. Those which do survive may emerge too weak or too few in number to effectively colonize the lower intestine. Moreover, recognizing the need for sound clinical studies in the area, some researchers express further concern as to the efficacy of certain lactobacillus species. Therefore, despite the disclosures relating to lactobacillus products, there still remains a need for improved urogenital and gastrointestinal tract compositions incorporating lactobacillus.

The present inventors have found that compositions incorporating plants or extracts of the Ericaceae family with microencapsulated Lactobacillus and/or Bifidobacterium provide improved compositions for treating and/or preventing urogenital and gastrointestinal disorders by modifying the interaction of pathogens with cellular tissue. Recent studies also suggest the value of Ericaceae extracts in treating urinary tract infections. Researchers have observed that various species of Vaccinium (e.g. cranberry and blueberry) contain a polymeric compound which inhibits the adhesion of common urinary pathogens (e.g., *E. coli*) to infection sites within the urinary tract. Ofek I et al., Anti-Escherichia coli Adhesin Activity of Cranberry and Blueberry Juices, N Engl J Med 1991;324:1599. Surprisingly, the compositions of the present invention provide improved environments more conducive to the colonization of lactic acid bacteria. Accordingly, it is an object of the present invention to provide compositions for dietary supplementation.

Another object of the present invention is to provide improved compositions comprising a viable colony of microencapsulated lactobacillus and/or bifidobacteria.

A further object of the present invention is to provide compositions effective in preventing and/or treating urogenital and intestinal disorders.

A still further object of the present invention is to provide topical compositions for vaginal use.

An even further object of the present invention is to provide methods for preventing and/or treating urogenital and intestinal disorders.

These and other objects will become readily apparent from the disclosure which follows.

### SUMMARY OF THE INVENTION

The present invention relates to compositions for the treatment or prevention of urogenital and intestinal disorders, comprising:

- a.) an antiadhesive amount of at least one plant species of the Ericaceae family or its extract; and

- b.) a viable culture of at least one species of microencapsulated bacteria selected from the group consisting of lactobacillus, bifidobacterium and mixtures thereof.

5 The present invention also relates to the above compositions further comprising a growth factor for facilitating the growth of lactic acid bacteria.

The phrase "urogenital and intestinal compositions," as used herein, means a product which in the ordinary course of usage may be retained in the oral cavity, swallowed or applied topically to provide urogenital and/or intestinal activity.

10 The phrase "antiadhesive amount," as used herein, means an amount effective to reduce the number of pathogenic microorganisms on the epithelial and/or mucosal lining of the urogenital and/or intestinal tract.

The term "urogenital," as used herein, means that system of organs concerned with the production and excretion of urine and reproduction.

The term "intestinal," as used herein, means of or relating to the intestines.

15 The term "nonpathogenic," as used herein, means substantially lacking the ability to cause disease or abnormality in healthy mammals. The term "healthy," as used herein, means free of underlying disease and/or immunosuppression.

All percentages and ratios used herein are by weight unless otherwise specified. Also, all measurements referred to herein are made at 25°C unless otherwise specified.

## 20 DETAILED DESCRIPTION OF THE INVENTION

The essential as well as optional components of the compositions of the present invention are described in the following paragraphs.

### ESSENTIAL COMPONENTS

#### Plants or extracts of the Family Ericaceae

25 The Ericaceae (heath) family, consisting of about 110 genera and 4,000 species, is by far the most important family of the Ericales order, encompassing a wide variety of fruit producing shrubbery and evergreen plants. Genera falling under Ericaceae family include Vaccinium, Arctostaphylos, Gaultheria, and Gaylussacia. The Arctostaphylos genus includes such species as the checkerberry and bearberry (*Uva ursi*). Other edible fruits  
30 such as the creeping snowberry or moxie plum fall under the genus Gaultheria. Huckleberries are a well known species of the genus Gaylussacia. The Vaccinium genus, best known for its fruits, contain some of the most common of berries, including the blueberry (e.g., *V. australe*), cranberry (e.g., *V. macrocarpon*) and bilberry (e.g., *V. myrtillus*). The term "berry (ies)," as used herein, means berries, drupes, plums and the  
35 like.

*E. coli* adherence results primarily from adhesins on the raised hair like fimbriae (or pili) of the microorganism. These adhesins are designated MS (mannose-sensitive) and MR (mannose-resistant). Like most fruit, Ericaceae fruit species contain fructose, an inhibitor of MS adhesins. However, it has been recently suggested that the plants or  
5 extracts of Ericaceae species further contain an unidentified, non-dialyzable polymeric compound which inhibits the MR adhesins associated with pyelonephritogenic strains of *E. Coli*. The unidentified polymeric compound, as studied in *Vaccinium* species, was found to inhibit the adhesion of both urinary and fecal isolates of *E. Coli*, the urinary isolates being inhibited to a greater extent. Ofek I et al., Anti-Escherichia coli Adhesin Activity of  
10 Cranberry and Blueberry Juices, N Engl J Med 1991;324:1599. It has also been reported that the ingestion of large quantities of cranberry juice increased the hippuric acid content of urine by several grams a day. This increase in hippuric acid excretion was accompanied by small decreases in urine pH. *In vivo* tests have established that hippuric acid was bacteriostatic at pH 5.0 for common pathogens of the urinary tract, but this action was  
15 considerably decreased as the urine pH was raised. Papas, N.P., et al., Cranberry Juice In the Treatment of Urinary Tract Infections, Southwestern Medicine, 47:No. 1 (Jan. 1966). That the Ericaceae species of the present invention are effective against other pathogenic bacteria (e.g., *Pseudomonas aeruginosa*) is disclosed in U.S. Patent 5,474,774, issued December 12, 1995, to Walker et al., herein incorporated by reference in its entirety; no  
20 effect was observed with respect to adhesion of lactobacillus strains to cells. Without being limited by theory, it is believed that the pathogenic inhibition caused by the Ericaceae plants or extracts results in decreased pathogenic interaction, providing a more favorable, less antagonistic environment for lactobacillus to initially adhere and maintain adherence. The phrase "anti-adhesive activity," as used herein, means an amount effective  
25 to inhibit the adhesion of pathogenic microorganisms to the epithelial and/or mucosal lining of the urogenital and/or intestinal tract.

Plants or extracts useful in the compositions of the present invention come from a wide range of Ericaceae genera including, but not limited to, *Vaccinium*, *Arctostaphylos*, *Gaultheria*, and *Gaylussacia*. Preferred species include, *V. australe*, *V. corymbosum*, *V. occidentale*, *V. ovatum*, *V. myrtillus*, *V. parvifolium*, *V. uliginosum*, *V. macrocarpon*, *V. oxycoccus*, *V. erythrocarpum*, *V. vitis-idaea*. *V. australe*, *V. macrocarpon*. *Vaccinium* species most preferred for use in the present invention include *V. australe*, *V. macrocarpon*, and *V. myrtillus*. Mixtures of Ericaceae plants and/or extracts may also be used.

The plants or extracts of the present invention are preferably concentrated, having a ratio of at least about 4 pounds of plant concentrate or extracts per pound of concentrate, more preferably from about 4 pounds of plant concentrates or extracts per pound of concentrate to about 50 pounds of plant concentrate or extracts per pound of concentrate.

5 The Ericaceae extracts are preferably present at a level of at least 10mg, more preferably from about 100mg to about 18g, most preferably from about 250mg to about 4g per unit dose. The amount of extract contained in each dose of product can be adjusted for the dosage form. For example, the amount of extract in powdered form used in a drink mix can range up to 18g per dose while the amount used in swallowable capsules might range

10 to about 4g. Preferred levels of the Ericaceae plants or extracts provide urinary and/or intestinal tract fluid concentrations of the above mentioned unidentified, non-dialyzable polymeric compound of from about 12 to about 25 micrograms per milliliter. Also, the plants or extracts of the present invention preferably retain greater than 2.5% of their total acid content and greater than about 0.1% of their benzoic acid content. The level is

15 selected to provide the desired level of anti-adhesin activity and can be modified as desired. Cranberries and cranberry extracts are useful in the treatment and/or prophylaxis of urinary tract infections and are also useful as vaginal deodorants.

#### Species of Lactobacillus or Bifidobacterium

Another essential component of the present invention is a viable colony of

20 microencapsulated Lactobacillus or Bifidobacterium. Bacteria of the Lactobacillus genus are characterized as rod-shaped, gram-positive and non-spore-forming bacteria. Of the family Lactobacillaceae, Lactobacillus inhabit the urogenital and gastrointestinal tracts of animals and humans and are important members of lactic acid producing group of bacteria. Various species of Lactobacillus are used commercially in the production of sour milks,

25 cheeses and yogurt. Lactobacilli also share an important role in the manufacture of fermented vegetables (e.g., pickles and sauerkraut), beverages (e.g., beer, wine and juices), sourdough breads, and some sausages.

Lactobacillus species suitable for use in the present invention are those which 1.) readily adhere to the epithelial cells of either the urogenital or gastrointestinal tracts of

30 mammals; 2.) produce hydrogen peroxide; 3.) promote low pH; and produce bacteriocins. By "bacteriocins," as used herein, means proteinaceous, bacteriocidal substances synthesized by bacteria, which usually have a narrow spectrum of activity, inhibiting strains of the same or closely related species. Bacteriocins appear to be capable of displacing or suppressing the growth of other bacteria, and as such may provide an

35 advantage to microorganisms in fermenting the female genital tract ecosystem. Preferred

species of *Lactobacillus* include *L. acidophilus*, *L. catenaforme*, *L. brevis*, *L. bulgaricus*, *L. lactis*, *L. reuterii*, *L. gasseri*, *L. helveticus*, *L. casei*, *L. plantarum*, *L. delbrueckii*, *L. thermophilis*, *L. jensenii*, *L. crispatus*, *L. rogosae* and *L. fermentum*. Species of *Lactobacillus* most preferred for use in the compositions of the present invention include  
5 *L. acidophilus*, *L. casei*, *L. crispatus*, *L. fermentum*, and *L. plantarum*. Preferably, the *Lactobacillus* species of the present invention are hydrogen peroxide producing such as *L. acidophilus*, *L. catenaforme*, *L. casei*, *L. crispatus*, *L. delbrueckii*, *L. jensenii*, *L. rogosae*, *L. fermentum*, *L. gasseri* and *L. plantarum* are also preferred for use herein in view of their adhesive properties.

10 Also inhabiting the urogenital and gastrointestinal tracts of mammals and useful to the compositions of the present invention are species of the genus *Bifidobacterium* (family Actinomycetaceae). *Bifidobacterium* species are non-acid-fast, nonmotile gram negative rods. Lactic and acetic acid producing *Bifidobacteria* are also considered important regulators of the urogenital and intestinal flora of mammals. Species suitable for use in the  
15 present compositions include, but are not limited to, *B. longum*, *B. breve*, *Lactobacillus Bifidus* and *Lactobacillus bifidus subsp pennsylvanicus*. Preferred for use in the present compositions is *B. Bifidum*, most preferred *B. Bifidum subsp. Pennsylvanicus*.

Mixtures of the *Lactobacillus* and/or *Bifidobacterium* species may also be used. Any of the above species may be obtained either commercially or through laboratory  
20 cultures.

The *Lactobacillus* and/or *Bifidobacterium* species are present as core and/or coating components at levels of at least about  $10^3$  cells per unit dose, preferably at levels of from about  $10^4$  to about  $10^{12}$  cells per unit dose and most preferably at levels of from about  $10^6$  to about  $10^{10}$  cells per unit dose. The phrase "unit dose," as used herein,  
25 means physically discrete units suitable as unitary dosages for administration to mammals, each such unit containing a predetermined quantity of an active ingredient calculated to produce the desired therapeutic effect in association with pharmaceutically acceptable carriers. The level is selected to provide the desired level of urogenital and gastrointestinal activity and can be modified as desired. *Lactobacillus* may lose 4-6 fold of its viability at  
30 room temperature and during manufacturing, so depending on the manufacturing conditions, an excess of *Lactobacillus* is added to maintain an adequate number of viable organisms per final unit dose form. Alternatively, a patient can be administered the equivalent of these concentrations of organisms where the values are expressed by some other measurement such as, for example, total protein concentration.



The Lactobacillus and/or Bifidobacterium species of the present invention must also be microencapsulated. Viable Lactobacillus and/or Bifidobacterium bacteria that have been lyophilized after the removal of the growth media can be used for encapsulation. The bacteria can be obtained from commercial sources, or can be obtained from laboratory strains. Suitable media include MRS, Thayer-Martin media, Trypticase Soy, Brain-Heart Infusion Broth, or any other enriched media suitable for the cultivation of these organisms, as no particular media is critical to the success of this suppository. The only important factors are the viability and quantity of the micro-organisms that are always determined by standard clinical laboratory dilution methods, such as plating the quantified dilution of bacteria on to blood agar plates or other enriched media, incubating at 37°C for 24-48 hours in a 5-10% carbon dioxide atmosphere, and then performing a colony count. The removal of the nutrient media is done by centrifugation at 14,000 x g at 0°-4°C., and then washing with sterile, balanced salts and 5% glucose solution at least three times after the initial centrifugation. The bacteria are then "snap frozen" with liquid nitrogen and then lyophilized under high vacuum. The bacteria are then microencapsuled according to conventional microencapsulation technology. Suitable methods of encapsulation are disclosed in U.S. Patent 5,466,463, issued November 14, 1995, to Ford and U.S. Patent 5,407,609, issued April 18, 1995, to Tice, both of which are herein incorporated by reference in their entirety.

#### OPTIONAL COMPONENTS

##### Lactobacillus Growth Factor

Also useful to the compositions of the present invention is a growth factor for facilitating the growth of lactic acid bacteria. The phrase "a growth factor for facilitating the growth of lactic acid bacteria," as used herein is meant a nutrient source or media which supplies a necessary source of food and/or energy for facilitating the growth of lactic acid producing bacteria. The growth factor is preferably selective for establishing and maintaining the growth of lactic acid bacteria, preferably Lactobacillus and/or Bifidobacterium, without facilitating extreme growth of pathogenic bacteria. The various nutritional requirements essential for bacterial and/or colony growth are normally met when the growth factor contain fermentable carbohydrate, peptone, meat and yeast extract. Supplementations with tomato juice, manganese, acetate and oleic acid esters, especially Tween 80, are stimulatory or even essential for most species and are, therefore, included in most MRS medium. Lactic acid bacteria adapted to very particular substrates may require special growth factors.

Examples of suitable growth factors include, but are not limited to, yeast extracts; gangliosides; salicin; mono-, di- and polysaccharide sugars such as glycogen, glucose, fructose, rhamnose, lactulose, methyl- $\alpha$ -D-mannoside, p-nitrophenol- $\alpha$ -D-mannoside, maltose, maltodextrin, dextrin, dextran, levan, sialic acid and acetylglucosamine as well as oligosaccharides such as, but not limited to, fructooligosaccharides, galactooligosaccharides and soybean oligosaccharides. Fiber or fermentable substrates such as psyllium may be used in the present compositions as may gums such as guar gum and xanthum gum. Similarly, proteinacious materials such as, peptone, keratin; vegetable; soy and unsaturated fatty acids such as lauric acid and teichoic acids such as lipoteichoic acid and esters such as glycerophosphates or  $\beta$ -glycerophosphates are also useful as growth factors. The growth factor is preferably selective for establishing and maintaining the growth of lactic acid bacteria, most preferably Lactobacillus and/or Bifidobacterium species. Growth factors preferable for use in the compositions of the present invention include lactose, lactulose, rhamnose, oligosaccharides and glycogen. Mixtures of these nutrients may also be used.

More preferably the growth factor of the present invention is an oligosaccharide such as, but not limited to, galactooligosaccharides, soybean oligosaccharides and fructooligosaccharides. Oligosaccharides possess bioadhesive properties which help fix the location of these growth factors for easier access by lactic acid bacteria. Most preferred for use herein are fructooligosaccharides. Lactic acid bacteria, such as Lactobacillus and Bifidobacterium, partially utilize fructooligosaccharides as an energy source by converting it, via fermentation, to lactic acid or a mixture of lactic acid, acetic acid, and CO<sub>2</sub>. The lactic acid and other fatty acids produced by this carbohydrate fermentation contribute to the maintenance of low pH which is an important control mechanism for preventing colonization of pathogens.

Chemically, oligofructose is the oligosaccharide fraction of inulin. It is composed of the GF<sub>n</sub> and F<sub>n</sub> type [G = glucose; F = fructose; n = number of fructose moieties linked by  $\beta$  (2,1) linkages in a ratio of about 2:1, with n = 2-6, and an average degree of polymerization of 4. Inulin is prepared by hot water extraction of chicory roots and is composed of molecules of the GF<sub>n</sub> type, n ranging as high as 60 with an average degree of polymerization of 10. Fructooligosaccharides suitable for use herein may or may not have non-fructosyl units in place of fructosyl end units. The same is true for other oligosaccharides with respect to their osyl end units. Non-fructosyl units may include, but are not limited to, polyalcohols such as xylitol, mannitol, and sorbitol.

Fructooligosaccharides most preferred for use in the present compositions are inulin or oligofructose. Mixtures of these nutrients may also be used.

Without being limited by theory, it is believed that, upon ingestion, growth factors increase the number of *Lactobacillus* and/or *Bifidobacterium* species available to displace pathogenic microorganisms from epithelial surfaces. The increase in the number of *Lactobacillus* and/or *Bifidobacterium* species competitively exclude the pathogens causing them to be displaced and excreted; the result is an overall reduction of host pathogens. Moreover, as vaginal infections are generally believed to result from pathogenic migrating from the rectum to the vagina, the number of pathogens invading the vagina are also reduced as the number of migratory pathogens decreases.

Growth factors are preferably incorporated into the compositions of the present invention at from about 5% to about 75%, more preferably from about 20% to about 70%, and most preferably from about 30% to about 65% per unit dose.

#### Coating Material

The compositions of the present invention may further comprise a coating material. Coating materials useful to the compositions of the present invention may be water soluble as well as water insoluble. The coating material of the present invention is preferably dried to a water activity ( $A_w$ ) of less than about 0.6, more preferably less than about 0.45, most preferably less than about 0.3. The term "water activity," as used herein, is well known in the art as the measure of the ratio of the equilibrium vapor pressure of water above a substance such as food (solid or liquid) to the vapor pressure of pure water, both taken at the same temperature. A more detailed description of water activity is found in U.K. Patent 2,014,429.

Water soluble coatings useful to the compositions of the present invention may include sugar or organic coatings. Sugar coatings useful to the present compositions may be syrupy materials containing monosaccharides or polymers of two or more saccharide units. Organic coatings are also useful to the compositions of the present invention. Useful organic polymers or copolymers used include those having a plurality of carboxylic acid and ester groups. Such groups are largely responsible for physical or chemical interactions with an active ingredient for effective taste masking properties. The preferred polymers or copolymers contain either a vinyl and acrylic acid and/or ester groups or carboxylic acid and/or ester groups. The specific polymers/copolymers are readily ascertained by one of ordinary skill in the art. Generally, polymers/copolymers that are pharmaceutically acceptable in terms of safety and toxicity may be used. It is preferred that such polymers/copolymers be soluble in a solvent or a mixture of solvents. Such

polymers/copolymers include polymeric or resinous substances such as: co-polymers of acrylic and substituted acrylic acids; cellulose esters; vinyl and substituted vinyl esters; polysulfonic acids, their esters and amides. Specific examples include naturally occurring materials such as shellac and zein and synthetic and semi-synthetic materials such as ethyl cellulose, cellulose acetates, cellulose acetate phthalates, ethyl vinyl acetates and/or phthalates, polyvinyl acetates and/or phthalates, ethyl and/or methyl methacrylic acids, esters and co-polymers, hydroxy alkyl cellulose acetates and/or phthalates. Such compounds include commercially available materials sold under trade names such as Eudragit S (trademark of Rohm Pharma) and Phthalavin (trademark of Colorcon). The coating material of the present invention is preferably a polymeric mixture of methacrylic acid and methacrylate. Mixtures of sugar and organic coating may also be used.

One particular embodiment of the present invention comprises a core containing at least one species of the Ericaceae family and a coating material containing a viable culture of at least one species of the above described microencapsulated bacteria. Alternatively, the microencapsulated Lactobacillus can reside in the core coated with a coating material containing the Ericaceae species. The latter may find use in providing an acidic environment for the Lactobacillus to survive and grow. Additional coating layers may also be added.

Additionally, Protein-like coating components may be included. Useful for improvement of gastrointestinal disorders, such components may contain branched amino acid-modified proteins. Whey powders, for example, are treated with papain in the presence of the amino acids ethyl L-leucine (16.1 parts), ethyl L-isoleucine (7.4 parts), ethyl L-valine (10.2 parts), cysteine hydrochloride (1.5 parts), and sodium carbonate (26 parts) in water at 40°C for 20 minutes to manufacture coated powders containing 10% free amino acids and 43% branched amino acids. The branched amino acid-modified powders can be mixed with fats, dextrans, salts, vitamins, and the like to make tablets.

Other examples of a tablet coating materials are zeolites and clays to make tablets more palatable. Zeolites have found use as bacterial feed coatings for domestic animals. For example, in the domestic animal business, tiamulin fumarate is dissolved in methanol, supported on mordenite-type zeolite or starch, dried and further premixed with the supports to produce sustained-release, coated granules. Still other examples of tablet coatings include complex carbohydrate and inclusion complexes.

The sugar and/or organic coatings of the present invention may be applied by conventional means including mechanical methods such as pan coating, air suspension techniques, multiorifice centrifugal techniques and spray drying techniques as well as

physicochemical methods such as coacervation-phase separation. Coating procedures are more fully discussed in Remington's Pharmaceutical Sciences (Alfonso Gennarol, editor), 1666-1675 (1990), herein incorporated by reference.

#### Buffering Agents

5           The compositions of the present invention may also contain a buffering agent. For oral compositions, buffering to an acidic pH to enhance flavor may be done. For products used in the vaginal area, buffering agents suitable for use in the compositions of the present invention are those capable of maintaining a urogenital pH of about 3.0 to about 5.5. Any mild pharmaceutically acceptable acid, other than those found in the Ericaceae  
10 species disclosed herein, can be used. Suitable acids include boric acid, or organic acids such as quinnic acid, propionic acid, malic acid, pyruvic acid, hippuric acid, tartaric acid, sorbic acid, benzoic acid, lactic acid, ascorbic acid, citric acid, or acetic acid, in combination with their respective sodium or other pharmaceutically acceptable salt (to the extent necessary to achieve the desired pH). When buffered, the compositions of the  
15 present invention are preferably buffered to a pH range of from about 3.5 to about 5.0, preferably from about 3.7 to about 4.7, and preferably using lactic acid with sodium lactate or a combination lactic acid/sodium lactate and benzoic acid or lactic acid/sodium lactate and propionic acid.

#### Additional Plant Extracts

20           Additional therapeutic and/or medicinal plants or extracts may also be incorporated into the compositions of the present invention. Such plants or extracts include echinacea, allium, buchu, juniper ginseng, allicin, chlorella, algin and the like. Mixtures of these additional plants or extracts may also be used.

#### Nutritional Additives

25           Nutritional additives may also be incorporated into the compositions of the present invention. Such additives include, but are not limited to, proteins and carbohydrates other than those mentioned herein as growth factors, vitamins, minerals, amino acids such as glycine, phytochemicals and mixtures thereof. These additives may, alternatively, be first incorporated into the Lactobacillus and/or Bifidobacterium of the present invention.

#### Pharmaceutical Actives

30           The compositions of the present invention may also be used in combination with pharmaceutical actives. The pharmaceutical active is preferably selected from at least one of an analgesic agent and/or a gastrointestinal agent. When incorporating pharmaceutical actives, appropriate measures should be taken to avoid contact with the microorganisms of

the present invention. Such measures may include, but are not limited to, modifying the microencapsulation process as suggested by U.S. Patent 5,466,463 to Ford.

5 Examples of analgesics preferred for use in the present invention include acetaminophen, acetyl salicylic acid, indomethacin and optically active isomers or racemates of ibuprofen, naproxen, flurbiprofen, carprofen, tiaprofenic acid, cicloprofen, ketoprofen, ketorolac, etodolac, indomethacin, sulindac, fenoprofen, diclofenac, piroxicam, benzydome, nabumetone, their pharmaceutically acceptable salts and mixtures thereof.

10 Examples of gastrointestinal agents preferred for use in the present invention include anticholinergics including atropine, clidinium and dicyclomine; antacids including aluminum hydroxide, bismuth subsalicylate, bismuth subcitrate, simethicone, calcium carbonate and magaldrate; H<sub>2</sub>-receptor antagonists including cimetidine, famotidine, nizatidine and ranitidine; laxatives including: docusate, phenolphthalein and casanthrol; gastroprotectants including sucralfate and sucralfate humid gel; gastrokinetic agents  
15 including metoclopramide and cisapride; proton pump inhibitors including omeprazole and antidiarrheals including: diphenoxylate, kaolin pectin, attapulgit and loperamide.

#### Carrier Materials

The carriers into which the compositions of the present invention may be incorporated are many and varied and depend largely upon the end use of the  
20 compositions. These carriers are pharmaceutically acceptable and include orally acceptable as well as topical compositions. They may be completely inert or contain or may be other active ingredients, yet the carriers must be compatible with the herein disclosed compositions. The term "compatible," as used herein, means that the carrier components are capable of being commingled with the components of the present  
25 invention, and with each other, in a manner such that there is no interaction which would substantially reduce the activity or viability of the compositions under ordinary use situations. Carrier materials must, of course, be of sufficiently high purity and sufficiently low toxicity to render them suitable for administration to the human being treated. Preferably the compositions of the present invention comprise from about 0.01% to about 99.99% of  
30 one or more carrier materials.

Carriers suitable for topical administration of the present compositions include suppositories, vaginal tablets or capsules, ovules, creams, solutions for lavages, emulsions, foams, gels, liniments, oils and ointments, douches.

35 Creams, gels and other base formulations may be used in topical administration of the present compositions to, for example, male or female genitalia (including the vulva

and vagina) and are prepared according to conventional methods for semi-solid compositions using excipients like vaseline, paraffin, vaseline oil, vegetable oils, animal oils, solid and liquid synthetic glycerides, waxes, lanolin, lanolin alcohols, sorbitan esters, fatty alcohols, liquid/solid polyethylene glycols, propylene glycols, polyethylene, starch, acrylamides, methacrylamides, derivatives of cellulose and carboxyvinylpolymers.

Ovules, suppositories, vaginal capsules or tablets and effervescent tablets may also be useful in topical application of the prevention. Ovules are similar to suppositories, ovoidal shaped and the excipients mainly used are semi-synthetic glycerides and polyethylene glycols and optionally also emulsifiers and surfactants.

The vaginal capsules are gelatinous envelopes or sachets within which is subdivided the suspension which is generally anhydrous and contains liquid paraffin, vaseline, vegetable oils and semi-synthetic oils and thickening agents. The tablets, shaped suitably for vaginal use, contain as main excipients lactose, starch, polyvinylpyrrolidone, cellulose derivatives, magnesium stearate, glycol. The effervescent tablets contain chemical components (i.e. sodium bicarbonate with citric acid or tartaric acid), which are necessary to develop carbon dioxide in order to produce effervescence.

The compositions of the present invention may also be incorporated into and topically applied by woven or nonwoven fabric materials such as tissues, wipes, feminine napkins, panty liners, tampons, diapers, incontinent care products and the like. Preferred for use herein are nonwoven fabrics. Nonwoven fabrics suitable for incorporating the present compositions are described in U.S. Patent 4,891,227 to Thaman et al., herein incorporated by reference.

Oral dosage forms are also useful as carriers for the present invention. These dosage forms contain compatible solid or liquid filler diluents or encapsulating substances which are suitable for oral administration to a human or lower animal.

Liquid dosage forms for oral administration may comprise dissolving or suspending the compositions of the present invention in a potable liquid, such as sterile or purified water. Alternatively, liquid or dry oral administration forms can comprise an enterically coated capsule containing the dosage forms. Suitable forms include emulsions, suspensions, solutions, syrups, and elixirs containing inert diluents commonly used in the art, such as purified water, sugars, polysaccharides, silicate gels, gelatin, or an alcohol. These inert diluents do not actively participate in the therapeutic effect of the present invention. However, such liquid forms may require special care where free water is present with the *Lactobacillus* to prevent fermentation or degradation of the *Lactobacillus*. Besides the inert diluents, such compositions can also contain wetting agents, emulsifying

agents, suspending agents, as well as additional therapeutic actives. For a more detailed description of liquid and liquid-like dosage forms, see Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, Pa. (1990), pages 1519-1544, herein incorporated by reference.

5       Tablets can be compressed, molded, triturated, enteric-coated, sugar-coated, film-coated or multiple compressed, containing suitable binders, lubricants, diluents, disintegrating agents, and flow-inducing agents.

Also useful are soft or hard gelatin capsules. Preferably, the gelatin shell is essentially transparent so as to enhance the aesthetic qualities of the capsule. Soft and  
10       hard gelatin shells generally comprise gelatin, a plasticizer and water. The starting gelatin material generally used in the manufacture of these capsules is obtained by the partial hydrolysis of collagenous material. Gelatin suitable for capsule manufacture is commercially available from the Sigma Chemical Company, St. Louis, Mo.. One or more plasticizers is incorporated to produce a gelatin shell. Useful plasticizers of the present  
15       invention include glycerin, sorbitan, sorbitol, or similar low molecular weight polyols, and mixtures thereof.

Techniques and compositions for making solid oral dosage forms are described in Marshall, "Solid Oral Dosage Forms," Modern Pharmaceutics, Vol. 7, (Banker and Rhodes, editors), 359-427 (1979), incorporated by reference herein. Techniques and  
20       compositions for making tablets (compressed and molded), capsules (hard and soft gelatin), troches and pills are described in Remington's Pharmaceutical Sciences (Arthur Osol, editor), 1553-1593 (1980) and U.S. Patent 4,935,243, to Borkan et al., issued June 19, 1990; these two references being incorporated herein by reference in their entirety. Specific examples of pharmaceutically acceptable carriers and excipients that may be used  
25       to formulate oral dosage forms, are described in U.S. Patent 3,903,297, Robert, issued September 2, 1975, incorporated by reference herein.

Alternatively, the compositions of the present invention may be achieved by incorporating the compositions of the present invention into freeze-dried or lyophilized tablets. Freeze-drying or lyophilization facilitates disintegration of the composition by  
30       forming the dried composition into an open matrix network. In most cases, this results in rapid permeation by the aqueous media, promoting timely delivery of the product. Suitable methods of freeze drying are well known in the art and commonly employed. Any suitable conventional method of freeze-drying may be utilized. A preferable method of freezing and drying is to fast freeze the composition and then dry the composition to a  
35       final moisture content of about 2% to about 5%. Suitable methods of freeze-drying and



production are taught by U.S. Patent 4,642,903, February 17, 1987, to Davies, U.S. Patent 4,946,684, August 7, 1990, to Blank et al., U.S. Patents 4,305,502 and 4,371,516, issued December 15, 1981 and February 1, 1983 respectively, to Gregory et al., and U.S. Patent 5,188,825, February 23, 1993, to Iles et al.; which are all incorporated herein by reference.

Similarly, the compositions of the present invention may be vacuum dried. Vacuum drying involves at least the partial drying of compositions at temperatures above compositions' collapse temperature. Freeze drying, on the other hand, involves the drying of compositions at temperatures below the compositions' collapse temperature. Any suitable method of vacuum drying may be used. Suitable vacuum drying processes are described in U.S. Patent 5,298,261, to Pebley et al., issued March 29, 1994, herein incorporated by reference.

One other form of tableting technology that may be applicable to the present invention is a liquid/liquid extract developed by Janssen Pharmaceutica Inc. and is identified by the trade name Quicksolv™. This technology is fully described in U.S. Patent 5,215,756 herein incorporated by reference.

Other optional ingredients well known to the pharmacist's art may also be included in amounts generally known for these ingredients, for example, natural or artificial sweeteners, flavoring agents, colorants, perfuming agents, buffering agents and the like to provide a palatable and pleasant looking final product, antioxidants, for example, butylated hydroxy anisole or butylated hydroxy toluene, and preservatives, for example, methyl or propyl paraben, potassium sorbate, or sodium benzoate, to prolong and enhance shelf life. A preferred optional component is also caffeine.

Those of ordinary skill in the art will quickly realize other suitable ingredients, diluents and dosage forms, or will be able to ascertain such, using routine experimentation. Further, the administration of the various compositions can be carried out using standard techniques common to those of ordinary skill in the art.

#### Examples

The following examples further describe and demonstrate embodiments within the scope of the present invention. These examples are strictly given for illustration purposes and are not to be construed as limitations of the present invention, as many variations are possible without departing from the spirit and scope of the invention as set forth herein.

#### Example I

The Lactobacillus and/or Bifidobacterium species cultures can be freeze dried (or purchased freeze dried). To provide freeze dried cultures, an inoculum of Lactobacillus

and/or Bifidobacterium is grown in a sterile nutrient media (e.g. Trypticase Soy agar broth). The media is removed by centrifugation. The bacteria isolates are washed using a sterile balanced salt and 5% glucose solution. The bacteria are then "snap frozen" with liquid nitrogen and vacuum freeze dried. The freeze dried product is then checked for bacterial plate count and then diluted so that the plate count per unit dose composition is from about  $10^3$  to about  $10^{12}$ .

### Example II

The freshly obtained, washed and lyophilized bacteria obtained as described above are suspended in 10 ml of 5% glucose saline solution in such volume so as to obtain a heavy suspension of bacteria which contains between one to  $10^{10}$  organisms per ml, at 0-4°C. All of these procedures are performed in the 0-4°C temperature range unless otherwise noted, in order to maintain viability of the lactobacilli bacteria which at room temperature lose viability. The suspension of bacteria is rapidly, but gently, stirred while 0.2-0.4 ml of sodium alginate solution (1.5% weight by volume) is added. The above mixture is then transferred into a 4 liter round bottom flask by using a nitrogen stream through a sheathed 14 gauge needle. The 4 liter round bottom flask was previously washed with a 5% albumin solution, and thereafter heated for at least 10 hours at 65°C, and the needle and the tubing used in the process have also been treated this way.

Thereafter the above mixture is forced through a 30 gauge multi-beveled needle under pressure using a large syringe and nitrogen stream. Very small droplets are generated at the end of the needle which are dried by the nitrogen and air stream around the 30 gauge needle, and the droplets are collected in an aqueous solution of 1.3-2% calcium chloride where they gel. Thereafter, they are washed at least three times with 0.08-0.13% 2-(N-cyclohexyl-amino) ethanesulfonic acid (CHES) solution and 1.0-1.5% calcium chloride solution.

The gelled droplets or little spheres are further washed with at least a five fold excess of the 0.1% CHES 1.1% calcium chloride, and normal saline solution. The resultant spheres are then "snap frozen" in liquid nitrogen and then lyophilized. After these steps, the encapsulated organisms can be used in the formulations of the present invention.

### Example III

A tablet form of the present invention is made by combining the following components using conventional mixing and tableting technology.

Ingredient	% Weight
Concentrated Cranberry Extract	37.300%

	Lactobacillus <sup>1</sup>	29.900%
	K2932 (5% ethanol soln) <sup>2</sup>	13.400%
	Avicel (pH 101) <sup>3</sup>	13.400%
	Explotab <sup>4</sup>	1.8%
5	Talc	3.600%
	Magnesium stearate	0.600%

<sup>1</sup> Microencapsulated *Lactobacillus Caseii* var. *ramnosus* (108 cfu/g).

<sup>2</sup> Polyvinylpyrrolidone

<sup>3</sup> TM for microcrystalline cellulose, a highly purified particulate form of cellulose

10 <sup>4</sup> A brand of sodium starch glycolate

The cranberry extract is granulated with half the Avicel and the K2932 in ethanol. The granulation is then passed through a 12 mesh screen and dried at 120°F. The dried granulation mixture is passed through a 20 mesh screen. To the sieved granulation is added the lactobacillus, remaining Avicel, Explotab and talc and mixed until uniform.

15 Magnesium stearate is then added to the uniform mixture with mixing. The resultant granulation mixture is then compressed using conventional tableting processes.

#### Example IV

A oral capsule form of the present invention is made by combining the following components using conventional mixing technology.

20	Ingredient	% Weight
	Concentrated Cranberry Extract	29.900%
	Bifidobacterium <sup>1</sup>	15.000%
	fructooligosaccharide <sup>2</sup>	29.900%
	Avicel <sup>3</sup>	22.400%
25	Ac Di Sol <sup>4</sup>	2.800%

<sup>1</sup> Microencapsulated *Lactobacillus bifidus subsp pennsylvanicus* (108 cfu/g).

<sup>2</sup> Available as NutraFlora FOS from Golden Technologies Company, Inc.

<sup>3</sup> TM for microcrystalline cellulose, a highly purified particulate form of cellulose.

<sup>4</sup> A brand of a cross-linked form of sodium carboxymethylcellulose.

30 Combine and mix the cranberry extract, fructooligosaccharide, Lactobacillus culture, Avicel and Act Di Sol in a V-blender until uniform. Unit dose amounts of the resultant mixture is then placed into suitably sized hard gelatin capsules.

#### Example V

35 A topical gel form of the present invention is made by combining the following components using conventional mixing technology.

	Ingredient	% Weight
	Concentrated Cranberry Extract	0.50%
	Lactobacillus <sup>1</sup>	2.50%
	fructooligosaccharide <sup>2</sup>	6.00%
5	Polyacrylamide and C <sub>13-14</sub>	
	Isoparaffin and Laureth-7 <sup>3</sup>	4.00%
	PPG-14 Butylether	8.00%
	Water, Purified	q.s.

1 Microencapsulated *Lactobacillus Caseii* var. *rhamnosus* (108 cfu/g).

10 2 Available as NutraFlora FOS from Golden Technologies Company, Inc.

3 Available as Sepigel from Seppic Corporation.

Water is added to a suitable size container. While mixing at a moderate speed (300 rpm), the Polyacrylamide and C<sub>13-14</sub> Isoparaffin and Laureth-7 is added to the water to form a water phase. Separately, the PPG-14 Butyl ether is placed in a container and covered. Using a Lightnin' Mixer with a 3 blade paddle prop, the cranberry extract and fructooligosaccharide are added to the PPG-14 Butyl ether and mixed at a low speed (100 rpm) until the cranberry extract and fructooligosaccharide are dissolved. The lactobacillus culture is added to the water phase and mixed at low speed (100 rpm) until a uniform solution results. The PPG-14 Butyl ether is slowly added to the water phase to form a gel. The resulting gel is mixed at moderate speed until uniform.

15

20

WHAT IS CLAIMED IS:

1. A composition for treating or preventing urogenital and intestinal disorders, comprising:
  - a.) an antiadhesive amount of at least one plant species of the Ericaceae family or its extract; and
  - b.) a viable culture of at least one species of microencapsulated bacteria selected from the group consisting of lactobacillus, bifidobacterium and mixtures thereof.
2. A composition according to Claim 1, further comprising a growth factor wherein the growth factor is selected from the group consisting of lactulose, rhamnose, oligosaccharides, glycogen and mixtures thereof.
3. A composition according to Claim 1 or 2, further comprising a coating material having an  $A_w$  of less than about 0.6 and having suspended therein the microencapsulated bacteria.
4. A composition according to any one of the preceding Claims, further comprising a carrier selected from group consisting of suppository, tablet, troche, oral liquid, suspension, capsule, gelatin capsule.
5. A composition according to any one of the preceding Claims, wherein the species of lactobacillus is selected from the group consisting of *L. acidophilus*, *L. gasseri*, *L. cateniforme*, *L. casei*, *L. crispatus*, *L. delbrueckii*, *L. jensenii*, *L. rogosae*, *L. fermentum*, *L. plantarum* and mixtures thereof and wherein the species of bacteria is present at a level of  $10^4$  to  $10^{12}$  lactobacillus cells per unit dose of the composition, preferably  $10^6$  to  $10^{10}$  lactobacillus cells per unit dose of the composition.
6. A composition according to any one of the preceding Claims, wherein the plant or extract is a species selected from the genus *Vaccinium*.
7. A composition according to any one of the preceding Claims, further comprising nutritional additives selected from the group consisting of vitamins, minerals,

amino acids, phytochemicals and mixtures thereof and/or an additional plant extract selected from the group consisting of echinacea, allium, buchu, juniper ginseng, allicin, chlorella, algin and mixtures thereof and/or a pharmaceutical active selected from the group consisting of analgesics, gastrointestinal actives and mixtures thereof.

8. A topically administered composition for treating or preventing urogenital disorders, comprising:
  - a.) an antiadhesive amount of at least one plant species of the Ericaceae family or its extract; and
  - b.) a viable culture of at least one species of microencapsulated bacteria selected from the group consisting of lactobacillus, bifidobacterium and mixtures thereof.
9. A composition according to Claim 8, further comprising an oligosaccharide growth factor selected from the group consisting of galactooligosaccharides, soybean oligosaccharides, fructooligosaccharides and mixtures thereof and/or a buffering agent selected from the group consisting of boric acid, hippuric acid, tartaric acid, sorbic acid, benzoic acid, lactic acid, ascorbic acid, citric acid, acetic acid, propionic acid, pharmaceutically acceptable salts thereof and mixtures thereof.
10. A composition according to Claim 8 or 9, further comprising a carrier selected from group consisting of suppository, vaginal tablet, vaginal gelatin capsules, vaginal troche, cream, gel, ointment, lotion, irrigant, douche, tissue, wipe, panty liner, feminine napkin, tampon, diaper and incontinent care product.

# INTERNATIONAL SEARCH REPORT

Internat. J. Application No  
PCT/US 97/01662

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 A61K35/78 //(A61K35/78,35:74)

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BULLETIN OF THE INTERNATIONAL DAIRY FEDERATION, vol. 313, 1996, pages 43-48, XP000677675 T. MIZOTA: "5.LACTULOSE AS A GROWTH PROMOTING FACTOR FOR BIFIDOBACTERIUM AND ITS PHYSIOLOGICAL ASPECTS" see the whole document ---	1,2
X	CA 1 298 556 A (BRUCE ANDREW W ;REID GREGOR (CA)) 7 April 1992 cited in the application see page 2, line 7 - page 5, line 5 ---	1,2
X	EP 0 122 104 A (ROBERTS JAMES GORDEN) 17 October 1984 see page 5, line 25 - page 8, line 5 see page 16, line 9 - page 19, line 11 --- -/-	1,5-8

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*A\* document member of the same patent family

Date of the actual completion of the international search

2 July 1997

Date of mailing of the international search report

15. 07. 97

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Rempp, G

# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 97/01662

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	US 5 176 911 A (TOSI SILVANA ET AL) 5 January 1993 cited in the application see column 1, line 27 - column 3, line 66 ---	1,2,4,5, 8,9
X	EP 0 415 720 A (JAPAN MAIZE PROD) 6 March 1991 see page 5, line 25 - line 37 see page 6, line 4 - line 10 ---	1,2,5,8, 9
X,P	US 5 525 341 A (WALKER EDWARD B ET AL) 11 June 1996 see column 1, line 50 - column 2, line 61 ---	1
A	WO 93 09793 A (REID GREGOR ;BRUCE ANDREW WATT (CA)) 27 May 1993 cited in the application ---	
A	DE 24 21 066 A (SOLCO BASEL AG) 25 March 1976 ---	
A	WO 84 03216 A (VIRDALM CARL AXEL) 30 August 1984 -----	



# INTERNATIONAL SEARCH REPORT

1. national application No.

PCT/US 97/01662

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claim(s) 8-10  
is(are) directed to a method of treatment of the human/animal  
body, the search has been carried out and based on the alleged  
effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such  
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all  
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment  
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report  
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is  
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search-fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat

Application No

PCT/US 97/01662

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